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16. ABSTRACT

Soybeans Glycine max(L.) cv. Essex were hydroponically grown in a greenhouse at 2 levels of ultraviolet-B(UV-B) radiation (0 and 2500 J m^{-2} day^{-1} biologically effective UV-B radiation) and 4 levels of P(6.5, 13, 26 and 52 μM). Plants were grown in each treatment combination to the complete expansion of the 4th trifoliolate leaf. UV-B radiation and reduced P supply generally decreased plant height, leaf area and total biomass, but increased specific leaf weight and flavonoid content (measured as absorbance of methanolic extracts). Although both UV-B radiation and low P supply produced deleterious effects on plant biomass, the effects were non-additive. The combination of UV-B and the lowest P level (6.5 μM) had no effect on total biomass or leaf area. This was at least partially due to the accumulation of flavonoids and leaf thickening. The results show that the sensitivity of soybean to UV-B radiation is dependent upon plant P supply. Plants experiencing P deficiency are less sensitive to UV-B than plants at optimum P levels.

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Effects of ultraviolet-B irradiance on soybean. VI. Influence of phosphorus nutrition on growth and flavonoid content

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Soybeans *Glycine max* (L.) cv. Essex were hydroponically grown in a greenhouse at 2 levels of ultraviolet-B (UV-B) radiation (0 and 2 500 J m^{-2} day^{-1} biologically effective UV-B radiation) and 4 levels of P (6.5, 13, 26 and 52 μM). Plants were grown in each treatment combination to the complete expansion of the 4th trifoliolate leaf. UV-B radiation and reduced P supply generally decreased plant height, leaf area and total biomass, but increased specific leaf weight and flavonoid content (measured as absorbance of methanolic extracts). Although both UV-B radiation and low P supply produced deleterious effects on plant biomass, the effects were non-additive. The combination of UV-B and the lowest P level (6.5 μM) had no effect on total biomass or leaf area. This was at least partially due to the accumulation of flavonoids and leaf thickening. The results show that the sensitivity of soybean to UV-B radiation is dependent upon plant P supply. Plants experiencing P deficiency are less sensitive to UV-B than plants at optimum P levels.

Additional key words – *Glycine max*, ozone depletion, stress interactions.

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Introduction

The current estimate of stratospheric ozone depletion due to atmospheric pollution by chlorofluorocarbons is approximately 2 to 4% (National Academy of Sciences 1984). Caldwell (1977) estimated that a 1% decrease in ozone concentration would result in an approximate 2% increase in biologically effective ultraviolet-B ($UV\text{-}B_{BE}$) radiation at temperate latitudes. Therefore, the current estimated decrease in stratospheric ozone concentration would result in up to an 8% increase in $UV\text{-}B_{BE}$ radiation.

Most studies concerning the effects of UV-B radiation on plants have been conducted under optimal growth conditions in growth chambers or greenhouses. Plants growing under natural conditions often experience some degree of environmental stress, due to the shortage of water and/or nutrients. A combination of stresses can be either antagonistic, synergistic, or independent in its effect on physiological or morphological growth characteristics. For example, Bogenrieder and Douté (1982) found that in *Lactuca sativa* and *Rumex alpinus*, reductions in dry matter due to UV-B radiation were increased at low total supply of all nutrients compared to high total nutrient supply. On the other hand, Teramura et al. (1984) found no significant difference in dry matter production in soybean due to UV-B radiation under water stressed and non-stressed conditions. The objective of this study was to investigate the influence of UV-B radiation on the growth of soybean at various P nutrition levels.

Abbreviations- UV-B, ultraviolet radiation between 280–320 nm; $UV\text{-}B_{BE}$, biologically effective ultraviolet radiation; $UV\text{-}B_{DNA}$, DNA damaging ultraviolet radiation; SLW, specific leaf weight.

Materials and methods

Soybean seeds *Glycine max* (L.) Merr. cv. Essex were sown in flats with Metromix 300 potting mixture (com-

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posted pine bark, peat moss, vermiculite, perlite, granite and sand) and grown from January to March, 1984 in greenhouses at the Univ. of Maryland, College Park, MD, USA. The natural photoperiod ranged from 10 to 11.5 h. The greenhouse day temperature ranged from 21° to 30°C and night temperature varied between 17° and 21°C. Midday levels of photosynthetically active radiation averaged between 1.0 to 1.2 mmol m^{-2} s^{-1} measured with a LI-COR quantum sensor. A week after germination, 96 plants were transferred to styrofoam sheets after washing the roots in deionized water (12 plants per treatment). Plants were secured in the holes with cotton plugs and placed in a dark plastic container with 6 l of nutrient solution. Styrofoam in contact with the nutrient solution was covered with black polyethylene to reduce light penetration and roots were aerated using a small aquarium air pump. Plants were grown in pH 6.5 ± 0.2 at 4 levels of phosphorus: 52, 26, 13, and 6.5 μM. Concentrations of other nutrients in the nutrient solution were: 1.33 mM NO_3^-; 0.44 mM NH_4^+; 0.92 mM K^+; 0.45 mM Ca^{2+}; 0.26 mM Mg^{2+}; 0.25 mM SO_4^{2-}; 0.3 μM Fe^{2+}; 1.62 μM H_3BO_3; 0.13 μM Mn^{2+}; 0.01 μM MoO_4^{2-}; 0.05 $\mu M$$Cu^{2+}$; and 0.07 μM Zn^{2+}. On alternate days 8 μM of $FeSO_4.7H_2O$ was added. The nutrient solution was renewed every 4 days. Plants were irradiated daily with UV-B radiation beginning 4 days after the transfer to the nutrient solution. The experiment was a two-way factorial design with 2 levels of factor A (UV-B) and 4 levels of factor B (P) with 12 plants in each treatment.

UV-B radiation was supplied by Westinghouse FS-40 sunlamps held in lamp frames suspended above the plants following the procedure of Mirecki and Teramura (1984). Lamps were filtered with either pre-solarized 0.08 mm cellulose acetate (transmission down to 290 nm) for the UV-B treatment, or 0.13 mm Mylar Type S plastic film (absorbs almost all radiation below 320 nm) as a control. The radiation filtered through cellulose acetate supplied a weighted irradiance of 2 500 J m^{-2} day^{-1} UV-B_{BE} using the generalized plant response action spectrum (Caldwell 1971) normalized at 280 nm. This is equivalent to 170 J m^{-2} day^{-1} UV-B_{DNA} when weighted according to the DNA action spectrum (Setlow 1974). The weighted irradiance of Mylar filtered lamps was 0 for both UV-B_{BE} and UV-B_{DNA}. The spectral irradiance of the lamps was the same as given by Teramura et al. (1984) and plants were irradiated for 6 h daily for a total of 25 days.

Leaf discs of 100 mm^2 were taken from the first 3 fully expanded trifoliolate leaves from 5 plants and extracted in 10 ml acidified methanol for flavonoid measurement, according to procedures of Mirecki and Teramura (1984). Extract absorbance at 305 nm measured on a Perkin-Elmer Model 124 double beam grating spectrophotometer was used as a measure of flavonoids.

Plants were harvested when the 4th trifoliolate leaf was fully expanded (V5 stage of development according to terminology of Fehr and Caviness 1977). At harvest, total dry weight, leaf area and plant height were determined.

Data were analyzed by a two factor analysis of variance. Significantly different means were separated at the 5% level using Newman-Keuls multiple range test (Zar 1974).

Results

Plant height was significantly reduced by UV-B radiation and decreased P supply (Tab. 1). The combination of UV-B and the lowest P level produced the greatest reduction in plant height. Plant biomass and leaf area were also significantly reduced by UV-B radiation (Tab. 1), but the magnitude of this effect was dependent upon P nutrition. At the lowest P supply no significant difference was observed, yet at higher P supplies a UV-B effect was discernible. Decreased P supply reduced both total biomass and leaf area under UV-B irradiated and non-irradiated conditions.

Tab 1. Plant height, dry weight, leaf area, specific leaf weight (SLW) and methanolic extract absorbance at 305 nm of UV-B irradiated and non-irratiated Essex soybeans grown in 4 levels of P for 25 days. Each value is the mean of 12 plants ±1 *SE*, except methanolic absorbance which is the mean of 15 measurements ±1 *SE*. Means followed by same letter are not significantly different at $P<0.05$ according to Newmann-Keuls multiple range test. DW, dry weight.

Phosphorus (μM)	Plant height (mm)	Total DW (g)	Leaf area (cm^{-2})	SLW ($g\ m^{-2}$)	Absorbance (305 nm)
		Control plants (0 J m^{-2} day^{-1})			
52.0	459 ±5 e	1.91 ±0.06 c	520 ±20 e	17.6 ±0.2 e	0.32 ±0.008 a
26.0	433 ±7 b	1.60 ±0.06 b	410 ±10 d	18.0 ±0.3 ae	0.35 ±0.005 b
13.0	429 ±5 b	1.50 ±0.05 b	340 ±10 c	19.3 ±0.4 ab	0.38 ±0.01 bc
6.5	362 ±6 cd	1.20 ±0.06 a	250 ±10 ab	20.4 ±0.6 b	0.40 ±0.008 cd
		UV-B irradiated plants (2 500 J m^{-2} day^{-1})			
52.0	389 ±3 a	1.62 ±0.07 b	420 ±10 d	19.0 ±0.1 ac	0.45 ±0.01 e
26.0	377 ±6 ac	1.43 ±0.06 b	360 ±10 c	18.8 ±0.3 a	0.48 ±0.02 ef
13.0	352 ±5 d	1.22 ±0.05 a	280 ±10 b	20.2 ±0.4 bc	0.49 ±0.02 f
6.5	326 ±4 f	1.15 ±0.04 a	220 ±10 a	22.7 ±0.4 d	0.54 ±0.02 f

Specific leaf weight (SLW) increased in response to UV-B radiation, but this effect was modified by P supply (Tab. 1). At high P supplies, UV-B radiation did not affect SLW while at lower P levels SLW increased with UV-B radiation. Reduced P supply increased SLW both under UV-B irradiated and non-irradiated conditions.

UV-B radiation and decreased P supply both significantly increased methanolic leaf extract absorbance (Tab. 1). The combination of UV-B radiation and the lowest P level produced the maximum absorbance, while absorbances decreased with increasing P level.

Discussion

The daily dose of UV-B radiation used in the study, when weighted for plant effectiveness (Caldwell 1971), was equivalent to that which would be received at College Park (39°N) under clear sky conditions on 21 June with an anticipated 16% stratospheric ozone depletion (Green et al. 1980). This UV-B dose produced significant changes in plant height, total leaf area and total biomass. UV-B irradiated plants were stunted and produced a smaller leaf area and less dry matter than non-irradiated plants. Reduction in biomass due to UV-B radiation alone averaged 12%. These effects are similar to those found in other investigations on soybean (Teramura 1980, Teramura et al. 1980, Vu et al. 1981). Like UV-B radiation, P deficiency also stunted growth and reduced leaf area, resulting in lower total dry matter. The average reduction in biomass due to P deficiency was 23%.

Although UV-B radiation and low P level both had deleterious effects on plant biomass, the effects were non-additive. The reductions in biomass due to P deficiency in UV-B irradiated treatments (average 21%) were less than that of non-irradiated treatments (average 25%). At maximum P deficiency the biomass reduction was greater in control (37%) than in UV-B treated plants (29%).

Both decreased P supply and UV-B radiation significantly increased leaf flavonoid content and SLW. Changes in SLW have been shown to be related to anatomical changes in leaf thickness in soybean (Mirecki and Teramura 1984). The combination of UV-B and the lowest P supply resulted in maximum flavonoid accumulation and increases in leaf thickness which may have ameliorated the effectiveness of UV-B radiation. Other investigators have also shown an increase in flavonoids and leaf thickness in UV-B irradiated plants (Mirecki and Teramura 1984, Robberecht and Caldwell 1983, Wellmann 1975). They suggest that these responses serve as possible UV-B protective mechanisms since flavonoids have high UV-B absorption properties, and increasing leaf thickness places a greater proportion of UV sensitive organelles into deeper, more protected layers.

In summary, the results of this study show that the sensitivity of soybean to UV-B radiation was dependent upon plant P supply. Plants experiencing P deficiency are less sensitive to UV-B than plants at optimum P levels. This reduced sensitivity is associated with increased flavonoid content, leaf thickening and possibly other morpho-physiological processes. The ecological and agricultural significance of these findings is that enhanced levels of UV-B radiation resulting from a stratospheric ozone depletion could potentially have greater effects on plants grown under high fertility conditions, i.e., well-fertilized agricultural regions.

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